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CHEMICAL EXAMINATION OF THE SEEDS OF *MATTHIOLA INCANA* (RED VARIETY)

PART I — STUDIES ON MUCILAGE

By

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(Received on 16th March, 1959)

The chemical examination of red variety of the seeds of *Matthiola incana* (Hindi-Lal Towdri) N. O. *Cruciferae* revealed the presence of an oil, a mucilage, a mixture of colouring matters and an essential oil. The studies on the mucilage have been described in the present communication.

The freshly extracted pure mucilage was dirty white in colour and fibrous in nature. The addition of 1-2% of acetic acid increased the yield of mucilage and it also did not hydrolyse the mucilage during extraction. On complete incineration the mucilage gave 4 25% of greyish white ash which on analysis was found to contain magnesium (1 05%) and iron (1·8%). The hydrolysis of the mucilage with 3% oxalic acid indicated the presence of xylose, galactose and aldobionic acid. The total percentage of the reducing sugars was 19·2%. After being hydrolysed with 3% oxalic acid, the mucilage was hydrolysed with 2-N sulphuric acid when the presence of xylose, galactose, rhamnose and galactouronic acid were detected. The total percentage of the reducing sugars in the hydrolysate was 22·3%. The mucilage, left after being hydrolysed with oxalic and 2-N sulphuric acids, was hydrolysed with conc. sulphuric acid when only glucose was obtained. The percentage of the reducing sugars was 20·82%.

Extraction of the mucilage :

Six hundred gms. of the dried powdered seeds were exhaustively extracted with petroleum ether (40-60°C) and acetone to remove oil, colouring matters and other organic impurities. The dried seeds were soaked in distilled water (61). Glacial acetic acid (30 ccs) was added to the mixture. The contents of the container were then stirred vigorously with a mechanical stirrer for 24-26 hours and filtered through a muslin cloth. The filtrate was added to rectified spirit when a stringy mass was obtained. It was seprated by centrifugal method (Yield. 81·6 gms; 13·6%).

Purification of the mucilage :

The crude mucilage was first dissolved in hot distilled water acidified with acetic acid (5 cc of acetic acid in 100 cc of distilled water) and was then filtered. The filtrate was added to the absolute alcohol and the mixture was stirred vigorously when the mucilage was precipitated out. It was taken out and dried. The dried mucilage was successively refluxed with petroleum ether, ether, benzene, chloroform and alcohol to remove all other organic substances which might be contaminated with it. Finally the pure mucilage was dried in vaccum.

The ultimate analysis of the pure mucilage gave :—

$C=40{\cdot}15\%$; $H=7{\cdot}22\%$ (rest will be oxygen and ash) (Equivalent weight was approximately 1180 by titration method).

Ash :—A weighed amount of mucilage was completely incinerated when 4·5% of a greyish white ash was obtained. Quantitative estimation of the ash gave iron= 1·8% and magnesium=1·05% in the mucilage.

Properties of the mucilage :

The mucilage was insoluble in all the organic solvents. The solubility of the mucilage in distilled water was very low (refrective index of the solution was found to be 1·3330 while that of the distilled water was 1·3336).

Hydrolysis of the Mucilage :

A. With Oxalic acid :—40·0 gms. of mucilage was taken in a pyrex beaker with 100 ccs. of distilled water and 3 gms. of oxalic acid. The mixture was heated in a water-bath for 24-26 hours. The test portion taken out from the reaction mixture at intervals showed that the hydrolysis started after a lapse of seven and half hours and took 24—26 hours for completion. The solution was filtered and the residue was washed.

The filtrate and the washings were neutralised with $BaCO_3$ when sugars acids were converted into soluble barium salts excess of oxalic acid being removed subsequently as barium oxalate. The solution was filtered and concentrated under reduced pressure. The syrup was shaken with methyl alcohol and filtered. The filtrate was tested for sugars and the residue for acids.

Sugars :—The methanol solution was freed from the solvent and the residue was taken in water. The aqueous solution was made upto 100 ccs. The

quantitative determination of sugars in the solution indicated that 19·2% of reducing sugars was present in the solution. The latter was examined chromatographically when the spots of xylose and arabinose were obtained.

The osazones were prepared and crystallised with alcohol when two osazones of m. p. s. 163-64°C and 200-01°C corresponding to phenylosazones of xylose and galactose were obtained.

Acids :—Barium in the barium salt of the sugar-acid was estimated quantitatively, percentage of which corresponded to the precentage of barium of the barium salt of aldobionic acid.

FOUND :—Ba= 15·89%; Calculated value for Ba of the Barium salt of aldobionic acid—16·18%.

Confirmation :—The barium salt was oxidised by concentrated nitric acid. The oxidation product was found to be mucic acid (m. p. =210-11°C).

2–N Sulphuric Acid :—The insoluble part of the mucilage left after separating water soluble products of oxalic acid hydrolysis was further hydrolysed by 2-N H_2SO_4. The product of hydrolysis began to reduce the Fehling's solution after a lapse of 5-6 hours and took 26-27 hours for complete hydrolysis. The hydrolysis products were washed up as above (vide supra) and the products after filtration were separated into sugars and sugar-acid portions while excess of sulphuric acid was removed as insoluble barium sulphate. The mucilage was kept for the hydrolysis with con. H_2SO_4.

Sugars :—The sugar solution made upto 100 ccs. was quantitatively estimated and 22·3% of reducing sugars were found to be present in the solution.

The chromatographic examination of the sugar solution indicated the presence of xylose, galactose and rhamnose. The presence of these sugars was confirmed by preparing their osazone derivatives when corresponding phenyl-osazones were obtained.

Acids :—The barium of the barium-salt of the acid was quantitatively estimated when the percentage of bairum corresponded to that of the barium-salt of galactouronic acid.

FOUND :—Ba=26·9%

With con. sulphuric acid :—The residue of the mucilage left after separating the water soluble products of 3% oxalic acid and 2-N sulphuric acids hydrolysis was finally hydrolysed with con. H_2SO_4. From hydrolysates glucose was isolated only. The presence of glucose was determined by chromatographic examination and confirmed by preparing its osazone derivative. The total percentage of the glucose was found to be 20·82%.

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DIFFUSION OF METAL AMMINE IONS THROUGH FILTER PAPER STRIPS FROM AQUEOUS SOLUTIONS OF METAL IONS CONTAINING VARYING QUANTITIES OF AMMONIA

By

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(Received on 5th May, 1959)

ABSTRACT

Filter paper strip chromatography has been employed to investigate the diffusion of metal ammine ions of copper (II) nickel (II), cadmium (II), zinc (II), cobalt (II) and silver (I) using 50% aqueous ethanol as solvent. The influence of varying the proportions of ammonia both in the presence and absence of ammonium chloride has been investigated. Increase in the concentration of ammonia results in a change in the R_F and R_M values. In the case of copper (II), and nickel (II) the values decrease with increasing concentration of ammonia, while in the other case the values increase. In general, with large excess of ammonia added the values tend to attain constancy.

The variation of the rate of diffusion of a metal ion due to complex formation is well known. Usually in chromatography, the complexing agent is added in the solvent and the movement of the complex ion through a column or filter paper is noted. But, these experiments create conditions, where only the influence of a large excess of the complexing agent may be observed. In a number of publications from these laboratories, Singh and Dey have studied the influence of the addition of the complexing agent to the solution and have thus been able to investigate the influence of varying proportions of the complexieg agent on the movement of the ions. They have investigated by this method, the behaviour of anionic complexes involving oxalates ([1,2]) citrates[3] and tartrates[4] of various metals and have also been able to suggest the separation of metals by formation of complex anions with citrates[5] and tartrates[6]. Not much work on the study of the behaviour of metal ammines appears to have been done. Miller and coworkers[7] studied some metal ammines in which the ammonia was added to the solvent, while Lederer[8] studied cobaltammine complexes by chromatography. The influence of varying concentration of ammonia on the diffusion of some metal ammine ions has also been studied by Singh and Dey[9].

In this paper we have described our detailed work on the movement of ammine complexes of copper (II), nickel (II), cadmium (II), zinc (II), cobalt (II) and silver (I) in the presence and absence of ammonium chloride.

EXPERIMENTAL

Solutions of 0·05M copper sulphate, nickel sulphate, cobalt sulphate, cadmium sulphate, zinc sulphate and silver nitrate were prepared using reagent grade chemicals and standardised as usual. Other reagents used were also chemically pure. Two series of mixtures were prepared. In the first series, mixtures containing a constant concentration of metal with varying proportions of ammonia, keeping the total volume constant were prepared. In a second series mixtures containing a constant concentration of metal, a fixed concentration of ammonium chloride and

varying proportions of ammonia were prepared keeping the total volume constant. Other sets of experiments were performed in which different amounts of ammonium chloride were added in each set. The solutions of the mixtures were spotted on strips 2 cm wide prepared from Whatman filter paper No. 1 and the solvent used was 50% aqueous ethanol. A simple ascending filter paper strip method was used as described by Gage, Douglass and Wender[10]. The chromatograms were run at constant temperature in a temperature controlled room at 24°C in all the casee, except in the case of Co^{++} and Zn^{++} where the temperature was 35°C. Time allowes was 90 minutes in each case. After running the chromatograms the strips werd dried and developed with reagents as described along with various tables. The R_F values were measured and the R_M values were also calculated from the relation:

$$R_M = \log \left(\frac{1}{R_F} - 1\right)$$

Cupric-Ammine Complex

The indicator used was a freshly prepared solution of potassium ferrocyanide.

TABLE I

No.	Ratio $Cu^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·60	0·17
2	1 : 0 : 8	0·06	- 1·20
3	1 : 0 : 10	0·06	- 1·20
4	1 : 0 : 12	0·06	- 1·20
5	1 : 0 : 14	0·08	- 1·06
6	1 : 0 : 16	0·08	- 1·06
7	1 : 0 : 18	0·08	- 1·06
8	1 : 0 : 20	0·08	- 1·06
9	1 : 0 : 22	0·09	- 1·00
10	1 : 0 : 24	0·09	- 1·00
11	1 : 0 : 28	0·09	- 1·00
12	1 : 0 : 36	0·10	- 0·95
13	1 : 0 : 40	0·16	- 0·72
14	1 : 0 : 60	0·16	- 0·72
15	1 : 0 : 80	0·17	- 0·68

Precipitation occurs with lower concentrations of ammonia but after the addition of 8 molar proportion of ammonia no precipitate is obtained. From table I it is seen that the R_F and R_M values suddenly decrease by the addition of ammonia, but as the concentrations of the same increases they slightly increase and go on increasing even upto 80 molar proportion of ammonia added.

TABLE II

No.	Ratio Cu^{+2} : NH_4Cl : NH_3	R value	- R_M value
1	1 : 0 : 0	0·60	0·17
2	1 : 8 : 0	0·34	- 0·29
3	1 : 8 : 4	0·32	- 0·33
4	1 : 8 : 6	0·29	- 0·39
5	1 : 8 : 8	0·22	- 0 55
6	1 : 8 : 10	0·22	- 0·55
7	1 : 8 : 12	0·22	- 0·55
8	1 : 8 : 14	0·22	- 0·55
9	1 : 8 : 16	0·22	- 0·55
10	1 : 8 : 18	0·22	- 0·55
11	1 : 8 : 20	0·22	- 0·55
12	1 : 8 : 22	0·22	- 0·55
13	1 : 8 : 24	0·22	- 0·55

It may be noted from the observations recorded above that the R_F and R_M values decrease by the addition of ammonium chloride and with the addition of ammonia there is a further decrease and continues with increasing concentrations of ammonia and finally becomes constant as the molar proportion of ammonia added is 8.

TABLE III

No.	Ratio Cu^{+2} : NH_4Cl : NH_3	R_F value	- R_M value
1	1 : 0 : 0	0·60	0·17
2	1 : 16 : 0	0·52	- 0 03
3	1 : 16 : 4	0·50	0·00
4	1 : 16 : 6	0·46	- 0·07
5	1 : 16 : 8	0·40	- 0·17
6	1 : 16 : 10	0·40	- 0·17
7	1 : 16 : 12	0·39	- 0·19
8	1 : 16 : 14	0·39	- 0·19
9	1 : 16 : 16	0·37	- 0·23
10	1 : 16 : 18	0·37	- 0·23
11	1 : 16 : 20	0·37	- 0·23
12	1 : 16 : 24	0·36	- 0·25
13	1 : 16 : 24	0·34	- 0·29
14	1 : 16 : 30	0·33	- 0·31
15	1 : 16 : 32	0·22	- 0·33
16	1 : 16 : 40	0·32	- 0·33

It may be noted from the observations recorded in Table III that with a higher proportion of ammonium chloride added the decrease in R_F value is less prominent. By the addition of ammonia the R_F and R_M values decrease constantly with increasing amounts of ammonia and finally attain constancy with a very large excess of ammonia.

TABLE IV

No.	Ratio $Cu^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·60	0·17
2	1 : 24 : 0	0·64	0·25
3	1 : 24 : 4	0·60	0·17
4	1 : 24 : 6	0·42	-0·14
5	1 : 24 : 8	0·39	-0·19
6	1 : 24 : 10	0·39	-0·19
7	1 : 24 : 12	0·39	-0·19
8	1 : 24 : 14	0·38	-0·21

As the molar proportion of ammonium chloride added is 24, the R_F and R_M values are found to increase. The values decrease with the addition of ammonia and tend to attain constancy with excess of ammonia.

TABLE V

No.	Ratio $Cu^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·60	0·17
2	1 : 32 : 0	0·66	0·29
3	1 : 32 : 4	0·59	0·16
4	1 : 32 : 6	0·57	0·12
5	1 : 32 : 8	0·56	0·10
6	1 : 32 : 10	0·52	0·03
7	1 : 32 : 12	0·53	0·05
8	1 : 32 : 14	0·53	0·05
9	1 : 32 : 16	0·52	0·03
10	1 : 32 : 18	0·52	0·03
11	1 : 32 : 20	0·50	0·00
12	1 : 32 : 22	0·50	0·00
13	1 : 32 : 24	0·49	-0·01

The observations made in Table IV is still confirmed in Table V i.e. with increase in the amount of ammonium chloride added, the R_F and R_M values increase. Then with the addition of ammonia the values diminish till they tend to become almost constant.

TABLE VI

No.	Ratio $Cu^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·60	0·17
2	1 : 40 : 0	0·71	0·39
3	1 : 40 : 4	0·61	0.19
4	1 : 40 : 6	0·61	0·19
5	1 : 40 : 8	0·62	0·21
6	1 : 40 : 10	0 62	0·21
7	1 : 40 : 11·2	0·62	0·21
8	1 : 40 : 12	0·62	0·21
9	1 : 40 : 14	0·62	0·21
10	1 : 40 : 16	0·62	0·21
11	1 : 40 : 18	0·62	0·21
12	1 : 40 : 20	0 62	0·21

The results are similar to those noted earlier, but in this case the values become constant even with 8 molar proportion of ammonia added.

Nickel-Ammine Complex.

The chromatograms were developed with dimethyl glyoxime.

TABLE VII

No.	Ratio $Ni^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 0 : 1·6	0·78	0·55
3	1 : 0 : 3·2	0·78	0·55
4	1 : 0 : 4·8	0·79	0·57
5	1 : 0 : 6·4	0 79	0·57
6	1 : 0 : 8	0·80	0·60
7	1 : 0 : 10	0·80	0·60
8	1 : 0 : 12	0·82	0·65
9	1 : 0 : 14	0·83	0 68
10	1 : 0 : 16	0·83	0·68
11	1 : 0 : 20	0·83	0 68
12	1 : 0 : 24	0·83	0·68
13	1 : 0 : 28	0·84	0·72
14	1 : 0 : 32	0·84	0·72
15	1 : 0 : 40	0·84	0·72
16	1 : 0 : 80	0·84	0·72

It may be seen from the values recorded in table VII that the R_F and R_M values do not change with small additions of the complexing agent, but gradually increase with increasing concentrations of the same. They become constant with the addition of 28 molar proportion of the complexing agent.

TABLE VIII

No.	Ratio $Ni^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 8 : 0	0·88	0·86
3	1 : 8 : 1·6	0·88	0 86
4	1 : 8 : 3·2	0·88	0·86
5	1 : 8 : 4·8	0·88	0·86
6	1 : 8 : 6·4	0·87	0·82
7	1 : 8 : 8	0·87	0·82
8	1 : 8 : 10	0·87	0·82
9	1 : 8 : 12	0.87	0·82
10	1 : 8 : 14	0.86	0·78
11	1 : 8 : 16	0.86	0·78
12	1 : 8 : 18	0.85	0·75
13	1 : 8 : 20	0·85	0·75

The addition of ammonium chloride increases the R_F value and it may also be seen from the values recorded that the R_F and R_M values do not change with small additions of ammonia, but gradually decrease with large increase in the concentrations of the same and finally have a tendancy to become constant.

TABLE IX

No.	Ratio $Ni^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 16 : 0	0·88	0·86
3	1 : 16 : 1·6	0·88	0·86
4	1 : 16 : 3·2	0·88	0·86
5	1 : 16 : 4·8	0·88	0·86
6	1 : 16 : 6·4	0·87	0·82
7	1 : 16 : 8	0·87	0·82
8	1 : 16 : 10	0·87	0·82
9	1 : 16 : 12	0·87	0·82
10	1 : 16 : 14	0·87	0·82
11	1 : 16 : 16	0·86	0·78
12	1 : 16 : 18	0·86	0·78
13	1 : 16 : 20	0·86	0·78

It may be noted from the observations recorded in Table IX that the R_F and R_M values behave in a similar manner as in Table VIII. The R and R_M values become constant with 16 molar proportion of the complexing agent.

TABLE X

No.	Ratio Ni^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 24 : 0	0 88	0·86
3	1 : 24 : 1·6	0·87	0 82
4	1 : 24 : 3·2	0·87	0·82
5	1 : 24 : 4·8	0·87	0·82
6	1 : 24 : 6·4	0·87	0·82
7	1 : 24 : 8	0·86	0·78
8	1 : 24 : 10	0·86	0·78
9	1 : 24 : 12	0·86	0·78
10	1 : 24 : 14	0·85	0·75
11	1 : 24 : 16	0 85	0·75
12	1 : 24 : 18	0·84	0·72
13	1 : 24 : 20	0·84	0·72
14	1 : 24 : 22	0·83	0 68

It may be noted from the observations recorded in Table X that the R_F and R_M values decrease with the addition of the complexing agent and continue to decrease with increasing the concentration of the same.

TABLE XI

No.	Ratio Ni^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 32 : 0	0·90	0·95
3	1 : 32 : 1·6	0 89	0·90
4	1 : 32 : 3·2	0·89	0·90
5	1 : 32 : 4·8	0·89	0 90
6	1 : 32 : 6·4	0·89	0 90
7	1 : 32 : 8	0·88	0·86
8	1 : 32 : 10	0·88	0·86
9	1 : 32 : 12	0·88	0·86
10	1 : 32 : 14	0 88	0·86
11	1 : 32 : 16	0·88	0·86
12	1 : 32 : 18	0·88	0·86
13	1 : 32 : 20	0·88	0·86
14	1 : 32 : 24	0·88	0·86
15	1 : 32 : 30	0·88	0 86
16	1 : 32 : 40	0·87	0·86
			0·82

The R_F and R_M values behave in a similar manner as in Table X. In this case R_F and R_M values become constant with 8 molar proportion of the complexing agent.

TABLE XII

No.	Ratio Ni^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·78	0·55
2	1 : 40 : 0	0·90	0·95
3	1 : 40 : 1 6	0·88	0·86
4	1 : 40 : 3·2	0·83	0·86
5	1 : 40 : 4·8	0 87	0·82
6	1 : 40 : 6·4	0·86	0·78
7	1 : 40 : 8	0·85	0 75
8	1 : 40 : 10	0·85	0·75
9	1 : 40 : 12	0·85	0·75
10	1 : 40 : 14	0·84	0·72
11	1 : 40 : 16	0·84	0·72
12	1 : 40 : 18	0·83	0·68
13	1 : 40 : 20	0 83	0·68

From Table XII it may be seen that the R_F and R_M values increase with the addition of ammonium chloride and the values decrease constantly with increasing the concentrations of ammonia. The values have a tendency to become constant with higher concentrations of ammonia. In the case of nickel (II), however, the concentration of ammonium chloride does not have a very marked effect on the R_F and R_M values as in the case of copper (II).

Cadmium—Ammine complex

The chromatograms were developed with H_2S water.

TABLE XIII

No.	Ratio Cd^{+3} : NH_4 Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·87	0·82
2	1 : 0 : 1·6	0·83	0·68
3	1 : 0 : 3·2	0·83	0·68
4	1 : 0 : 4·8	0·83	0·68
5	1 : 0 : 6·4	0·84	0·72
6	1 : 0 : 8	0·84	0·72
7	1 : 0 : 10	0·85	0·75
8	1 : 0 : 12	0·86	0·78
9	1 : 0 : 14	0·86	0·78
10	1 : 0 : 16	0·87	0·82
11	1 : 0 : 18	0·87	0·82
12	1 : 0 : 20	0·83	0·86
13	1 : 0 : 24	0·88	0·86
14	1 : 0 : 28	0·89	0·90
15	1 : 0 : 32	0·89	0·90
16	1 : 0 : 40	0·89	0·90
17	1 : 0 : 80	0·89	0·90

It may be noted from the observation recorded in Table XIII that the R_F and R_M values decrease initially with the addition of ammonia, but then increase constantly with increasing the concentrations of ammonia and finally become constant. The values become constant with 28 molar proportion of the com plexing agent.

TABLE XIV

No.	Ratio $Cd^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·87	0·82
2	1 : 8 : 0	0·82	0 65
3	1 : 8 : 1.6	0·83	0·68
4	1 : 8 : 3.2	0·83	0·68
5	1 : 8 : 4.8	0·83	0·68
6	1 : 8 : 6.4	0·83	0·68
7	1 : 8 : 8	0·84	0·72
8	1 : 8 : 10	0 84	0·72
9	1 : 8 : 12	0·84	0·72
10	1 : 8 : 14	0·85	0·75
11	1 : 8 : 16	0·85	0·75
12	1 : 8 : 18	0 85	0·75
13	1 : 8 : 20	0·87	0·82
14	1 : 8 : 24	0·89	0·90
15	1 : 8 : 32	0 89	0·90
16	1 : 8 : 40	0·90	0·95

It may be noted from Table XIV that the R_F and R_M values decrease with addition of ammonium chloride, but increase with the addition of ammonia and continue to increase with increasing the concentrations of the same. The R_F and R_M values respectively become 0·90 and 0·95 with 40 molar proportion of the complexing agent.

TABLE XV

No.	Ratio $Cd^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·87	0·82
2	1 : 16 : 0	0·82	0·65
3	1 : 16 : 1·6	0·84	0·72
4	1 : 16 : 3·2	0·84	0·72
5	1 : 16 : 4·8	0·84	0·72
6	1 : 16 : 6·4	0·84	0·72
7	1 : 16 : 8	0·84	0·72
8	1 : 16 : 10	0·84	0·72
9	1 : 16 : 12	0·85	0·75
10	1 : 16 : 14	0·86	0·78
11	1 : 16 : 16	0·86	0·78
12	1 : 16 : 18	0·86	0·78
13	1 : 16 : 20	0·87	0·82
14	1 : 16 : 24	0.87	0·82
15	1 : 16 : 32	0.88	0·85
16	1 : 16 : 40	0.88	0·86

The values are similar to those in Table XIV.

TABLE XVI

No.	Ratio Cd^{+2} : NH_4Cl : NH_3	R_F value	— R_M value
1	1 : 0 : 0	0·87	0·82
2	1 : 24 : 0	0·84	0·72
3	1 : 24 : 1·6	0·84	0·72
4	1 : 24 : 3·2	0·85	0·75
5	1 : 24 : 4·8	0·85	0·75
6	1 : 24 : 6·4	0·85	0·75
7	1 : 24 : 8	0·85	0·75
8	1 : 24 : 10	0·85	0·75
9	1 : 24 : 12	0·87	0·82
10	1 : 24 : 14	0·87	0·82
11	1 : 24 : 16	0·87	0·82
12	1 : 24 : 18	0.89	0·90
13	1 : 24 : 20	0.89	0·90
14	1 : 24 : 24	0·90	0·95
15	1 : 24 : 28	0·90	0·95
16	1 : 24 : 32	0·90	0 95

From Table XVI it can be seen that the R_F and R_M values do not change with small additions of ammonia and with larger increase in the concentrations of the same they also increase. Finally they become constant with 24 molar proportion of the complexing agent added.

TABLE XVII

No.	Ratio Cd^{+2} : NH_4Cl : NH_3	R_F value	— R_M value
1	1 : 0 : 0	0·87	0·82
2	1 : 32 : 0	0·86	0·78
3	1 : 32 : 1·6	0·86	0·78
4	1 : 32 : 3·2	0·86	0·78
5	1 : 32 : 4·8	0.86	0·78
6	1 : 32 : 6·4	0·86	0·78
7	1 : 32 : 8	0·87	0·82
8	1 : 32 : 10	0·87	0·82
9	1 : 32 : 12	0·87	0·82
10	1 : 32 : 14	0·88	0·86
11	1 : 32 : 16	0·88	0·86
12	1 : 32 : 18	0·89	0·90
13	1 : 32 : 20	0·90	0·95
14	1 : 32 : 24	0·91	1·00
15	1 : 32 : 32	0·91	1·00
16	1 : 32 : 40	0·91	1·00

From Table XVII it can be seen that the R_M and R_F values behave in a similar manner as in Table XVI. Here also the values become constant with 24 molar proportion of the complexing agent added.

TABLE XVIII

No.	Ratio Cd^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·87	0·82
2	1 : 40 : 0	0·88	0·86
3	1 : 40 : 1·6	0 88	0·86
4	1 : 80 : 3 2	0·88	0·86
5	1 : 40 : 4·8	0·89	0·90
6	1 : 40 : 6·4	0·89	0·90
7	1 : 40 : 8	0·90	0·95
8	1 : 40 : 10	0·91	1·00
9	1 : 40 : 12	0·91	1·00
10	1 : 40 : 14	0·91	1·00
11	1 : 40 : 16	0·91	1·00
12	1 : 40 : 18	0·91	1·00
13	1 : 40 : 20	0·91	1 00
14	1 : 40 : 24	0·91	1·00
15	1 : 40 : 32	0 91	1·00
16	1 : 40 : 40	0·91	1·00

The values do not change with the small additions of ammonia and then begin o increase as the concentrations of the same is largely increased and finally have a tendency to become constant. They become constant with 10 molar proportion of the complexing agent added. The addition of ammonium chloride lowers the R_F and R_M values but when 40 molar proportion of ammonium chloride is added the values have a tendency to increase

Zinc Ammine—complex

The indicator spray reagent was a freshly prepared solution of 8-hydroxy quinoline.

TABLE XIX

No.	Ratio Zn^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0 85	0·75
2	1 : 8 : 0	0·90	0·95
3	1 : 8 : 1·6	0·90	0·95
4	1 : 8 : 3·2	0·90	0·95
5	1 : 8 : 4·8	0·90	0·95
6	1 : 8 : 6·4	0·90	0·95
7	1 : 8 : 8	0·91	1·00
8	1 : 8 : 10	0·91	1·00
9	1 : 8 : 12	0·91	1·00
10	1 : 8 : 14	0 91	1·00
11	1 : 8 : 16	0·91	1·00
12	1 : 8 : 18	0·92	1·06
13	1 : 8 : 20	0 92	1·06
14	1 : 8 : 22	0·93	1·12
15	1 : 8 : 24	0·93	1·12

From Table XIX it is clear that the R_F and R_M values increase with the addition of ammonium chloride but do not change with small additions of ammonia

and with large increase in the concentratiens of the same they increase and finally become constant with the addition of 22 molar proportion of the comlexing agent.

TABLE XX

No.	Ratio Zn^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·85	0·75
2	1 : 16 : 0	0·93	1·12
3	1 : 16 : 1·6	0·93	1·12
4	1 : 16 : 3·2	0·93	1·12
5	1 : 16 : 4·8	0·93	1·12
6	1 : 16 : 6·4	0·93	1·12
7	1 : 16 : 8	0·93	1·12
8	1 : 16 : 10	0·94	1·19
9	1 : 16 : 12	0·94	1·19
10	1 : 16 : 14	0·94	1·19
11	1 : 16 : 16	0·94	1·19
12	1 : 16 : 18	0·95	1·27
13	1 : 16 : 20	0·95	1·27
14	1 : 16 : 22	0·95	1·27
15	1 : 16 : 24	0·95	1·27

As the amount of amonium chloride is further increased the R_F and R_M values increase. The addition of ammonia has a little effect except that the values have a tendency to increase slightly.

TABLE XXI

No.	Ratio Zn^{+2} : NH_4Cl : NH_3	R_F value	$-R_M$ value
1	1 : 0 : 0	0·85	0·75
2	1 : 24 ; 0	0·93	1·12
3	1 : 24 : 1·6	0·93	1·12
4	1 : 24 : 3·2	0·93	1·12
5	1 : 24 : 4·8	0·93	1·12
6	1 : 24 : 6·4	0·93	1·12
7	1 : 24 : 8	0·94	1·19
8	1 : 24 : 10	0·94	1·19
9	1 : 24 : 12	0·94	1·19
10	1 : 24 : 14	0·95	1·27
11	1 : 24 : 16	0·95	1·27
12	1 : 24 : 18	0·95	1·27
13	1 : 24 : 20	0·95	1·27
14	1 : 24 : 22	0·95	1·27
15	1 : 24 : 24	0·95	1·27

Further increase in the concentration of ammonium chloride has no effect on the diffusion. The addition of ammonia also produces results similar to those in Table XX.

TABLE XXII

No.	Ratio $Zn^{+2} : NH_4Cl : NH_3$	R_F value	$- R_M$ value
1	1 : 0 : 0	0·85	0·75
2	1 : 32 : 0	0·93	1·12
3	1 : 32 : 1·6	0·93	1·12
4	1 : 32 ; 3·2	0·93	1·12
5	1 : 32 : 4·8	0·94	1·19
6	1 : 32 : 6·4	0·94	1·19
7	1 : 32 : 8	0·94	1·19
8	1 : 32 : 10	0·94	1·19
9	1 : 32 : 12	0·94	1·19
10	1 : 32 : 14	0·94	1·19
11	1 : 32 : 16	0·95	1·27
12	1 : 32 : 18	0·95	1·27
13	1 : 32 : 20	0·95	1·27
14	1 : 32 : 22	0·96	1·38
15	1 : 32 : 24	0·96	1·38

From Table XXII it may be seen that the R_F and R_M values behave in a similar manner as in previous tables.

TABLE XXIII

No.	Ratio $Zn^{+2} : NH_4Cl : NH_3$	R_F value	$- R_M$ value
1	1 : 0 : 0	0·85	0·75
2	1 : 40 : 0	0·93	1·12
3	1 : 40 : 1·6	0·93	1·12
4	1 : 40 : 3·2	0·93	1·12
5	1 : 40 : 4·8	0·93	1·12
6	1 : 40 : 6·4	0·94	1·19
7	1 : 40 : 8	0·94	1·19
8	1 : 40 : 10	0·94	1·19
9	1 : 40 : 12	0·94	1·19
10	1 : 40 : 14	0·94	1·19
11	1 : 40 : 16	0·94	1·19
12	1 : 40 : 20	0·95	1·27
13	1 : 40 : 24	0·95	1·27
14	1 : 40 : 28	0·95	1·27

It may be noted from the observations recorded in Table XXIII that the R_F and R_M values increase with the addition of ammonium chloride, but tend to become constant as the amount of it is increased. The value do not change with the small additions of ammonia and they increase only slightly with increasing the concentrations of the same and finally become constant with 20 molar proportion of the complexing agent.

Cobalt—Ammine complex

H_2S water was used for developing.

TABLE XXIV

No.	Ratio $Co^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·89	0·90
2	1 : 8 : 0	0·91	1·00
3	1 : 8 : 1·6	0·92	1·06
4	1 : 8 : 3·2	0·92	1·06
5	1 : 8 : 4·8	0·92	1·06
6	1 : 8 : 6·4	0·93	1·06
7	1 : 8 : 8	0·93	1·12
8	1 : 8 : 10	0·93	1·12
9	1 : 8 : 12	0·93	1·12
10	1 : 8 : 14	0·93	1·12
11	1 : 8 : 16	0·94	1·12
12	1 : 8 : 18	0·94	1·19
13	1 : 8 : 20	0·94	1·19
14	1 : 8 : 24	0·95	1·19
15	1 : 8 : 28	0·95	1·27
16	1 : 8 : 32	0·95	1·27

It may be noted from Table XXIV that the R_F and R_M values increase with the addition of ammonium chloride and also with small additions of ammonia and continue to increase with increasing the concentration of the same. The values become constant with the addition of 24 molar proportion of the complexing agent.

TABLE XXV

No.	Ratio $Co^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·89	0·90
2	1 : 16 : 0	0·92	1·06
3	1 : 16 : 1·6	0·93	1·12
4	1 : 16 : 3·2	0·93	1·12
5	1 : 16 : 4·8	0·93	1·12
6	1 : 16 : 6·4	0·93	1·12
7	1 : 16 : 8	0·93	1·12
8	1 : 16 : 10	0·94	1·19
9	1 : 16 : 12	0·94	1·19
10	1 : 16 : 14	0·95	1·27
11	1 : 16 : 16	0·95	1·27
12	1 : 16 : 18	0·95	1·27
13	1 : 16 : 20	0·95	1·27
14	1 : 16 : 24	0·96	1·38
15	1 : 16 : 28	0·96	1·38
16	1 : 16 : 32	0·96	1·38

From Table XXV it can be seen that the R_M and R_F values behave in a similar manner as in Table XXIV. The values do not change up to the addition of

8 molar proportion of ammonia. They become constant with the addition of 24 molar proportion of the complexing agent.

TABLE XXVI

No.	Ratio $Co^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·89	0·90
2	1 : 24 : 0	0·92	1·06
3	1 i 24 : 1·6	0·93	1·12
4	1 : 24 : 3·2	0·93	1·12
5	1 : 24 : 4·8	0·93	1·12
6	1 : 24 : 6·4	0·93	1·12
7	1 : 24 : 8	0·94	1·19
8	1 : 24 : 10	0·94	1·19
9	1 : 21 : 12	0·94	1·19
10	1 : 24 : 14	0·94	1·19
11	1 : 24 : 16	0·94	1·19
12	1 : 24 : 18	0·95	1·27
13	1 : 24 : 20	0·95	1·27
14	1 : 24 : 24	0·95	1·27
15	1 : 24 : 28	0·95	1·27
16	1 : 24 : 32	0·95	1·27

From Table XXVI it may be seen that the results are similar as in Table XXIV. The R_F and R_M values become constant with the addition of 18 molar proportion of ammonia.

TABLE XXVII

No.	Ratio $Co^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·89	0 90
2	1 : 32 : 0	0·92	1·06
3	1 : 32 : 1·6	0·93	1·12
4	1 : 32 : 3·2	0·93	1·12
5	1 : 32 : 4·8	0·93	1·12
6	1 : 32 : 6·4	0·94	1·19
7	1 : 32 : 8	0·94	1·19
8	1 : 32 : 10	0·94	1·19
9	1 : 32 : 12	0·94	1·19
10	1 : 32 : 14	0·94	1·19
11	1 : 32 : 16	0·95	1·27
12	1 : 32 : 18	0·95	1·27
13	1 : 32 : 20	0·95	1·27
14	1 : 32 : 24	0·95	1·27
15	1 : 32 : 28	0·95	1·27
16	1 : 32 : 32	0·95	1·27

It may be noted from the observations recorded in Table XXVII that the R_F and R_M values behave in a similar manner as in Table XXIV. The R_F and R_M values attain constancy with the addition of 16 molar proportion of the complexing agent.

TABLE XXVIII

No.	Ratio $Co^{+2} : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·89	0·90
2	1 : 40 : 0	0·95	1·27
3	1 : 40 : 1·6	0·95	1·27
4	1 : 40 : 3·2	0·95	1·27
5	1 : 40 : 4·8	0·95	1·27
6	1 : 40 : 6·4	0·95	1·27
7	1 : 40 : 8	0·96	1·38
8	1 : 40 : 10	0·96	1·38
9	1 : 40 : 12	0 96	1·38
10	1 : 40 : 14	0·96	1·38
11	1 : 40 : 16	0·96	1·38
12	1 : 40 : 18	0·96	1·38
13	1 : 40 : 20	0·96	1·38
14	1 : 40 : 24	0·96	1·38

It may be noted from Table XXVIII that the R_F and R_M values increase with the addition of ammonium chloride. They do not change with the small additions of ammonia and then increase with larger increase in the concentrations of the same. The R_F and R_M values do not change up to the addition of 6·4 molar proportion of ammonia, and become constant with 8 molar proportion of the same.

Silver-Ammine complex

H_2S water was used for developing.

TABLE XXIX

No.	Ratio $Ag^+ : NH_4Cl : NH_3$	R_F value	$-R_M$ value
1	1 : 0 : 0	0·57	0·12
2	1 : 0 : 1·6	0·53	0·05
3	1 : 0 : 3·2	0·52	0·03
4	1 : 0 : 4·8	0·55	0·09
5	1 : 0 : 6·4	0·55	0·09
6	1 : 0 : 8	0·56	0·10
7	1 : 0 : 10	0·57	0·12
8	1 : 0 : 12	0·58	0·14
9	1 : 0 : 14	0·59	0·16
10	1 : 0 : 16	0·60	0·17
11	1 : 0 : 18	0·60	0·17
12	1 : 0 : 20	0·60	0·17
13	1 : 0 : 24	0·60	0·17
14	1 : 0 : 28	0·61	0·19
15	1 : 0 : 32	0·61	0·19
16	1 : 0 : 36	0·61	0·19
17	1 : 0 : 40	0·61	0·19

The movement of silver ammine ion has been studied with ammonia only. In the case of silver (I), however, ammonium chloride could not be used due to the precipitation of silver chloride. It is noted that with the addition of 1·6 to 3·2 equivalents of the complexing agent to the silver salt the R_F and R_M values decrease and a precipitate is obtained. By further increasing the concentrations of the complexing agent, the R_F and R_M values increase and become constant with 28 molar Proportion of the complexing agent.

From the results presented in the foregoing tables it might be of interest to record the influence of the addition of ammunium chloride to the solutions, on the R_F values of the metal ions. The values are summarised below;

TABLE XXX

	R_F values with ammonium choloride (molar proportion)						
Metal ion	0	8	16	24	32	40	80
Cu^{++}	0·60	0·34	0·52	0·64	0·66	0·71	0·75
Ni^{++}	0·78	0·88	0·88	0·88	0·90	0·90	0·91
Cd^{++}	0.87	0·82	0 82	0·84	0·86	0·88	0·92
Zn^{++}	0 85	0·90	0·93	0·93	0·93	0·93	0·94
Co^{++}	0 89	0·91	0·92	0·92	0·92	0·95	0·96

The addition of ammonium chloride has the most marked influence in the case of copper (II), when the R_F value diminishes at first, but then increase again as the amount of ammonium chloride is largerly increased. In other cases, however, the influence of the concentration of ammonium chloride is less marked. Further, it may also be seen that with no added ammonium chloride the R_F values in the case of all the metals tend to become constant with 28 molar proportion of ammonia added, except in copper (II) where even a large amount of ammonia is required for constancy of R_F and R_M values. From these studies it may be concluded that by a control of the concentration of ammonium chloride and ammonia added, it might be possible to use the complexing property of metals with ammonia for chromatographic analysis of the metals. Further work in this direction is, however, needed to utilise this for analytical work.

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KINETICS OF REDUCTION OF ALKALINE BIVALENT COPPER BY REDUCING SUGARS—PART VIII

By

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ABSTRACT

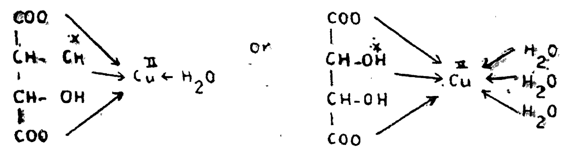
The rate of oxidation of aldehydo hexose or pentose by alkaline cupric-tartrate or citrate complex is the rate of enolization of sugar and the same is the case with keto sugars.

In previous publications[1,2,3] we have discussed that the rate of the reaction between alkaline bivalent copper in presence of Rochelle's salt and the reducing sugar is directly proportional to the concentration of reducing sugar and independent of the concentration of copper complex. The reaction is catalysed by the cuprous oxide produced in the reaction and the reaction velocity is also enhanced by increasing the concentration of hydroxyl ions but it is not directly proportional. It has also been reported that the previous treatment of the reducing sugar with alkali enhances the reaction rate. It has further been pointed in earlier papers that the minimum quantity of tartrate or citrate required for complete reduction of copper sulphate is equivalent to copper sulphate. Any additional quantity of tartrate or the citrate when added does not produce any appreciable change in the velocity constants. In the following lines we shall discuss in detail the function of tartrate and citrate ions.

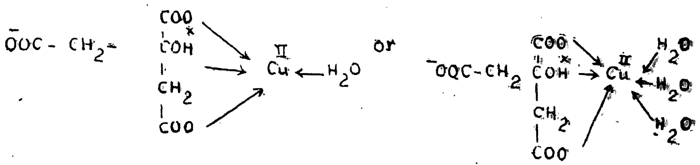
FUNCTION OF TARTRATE OR CITRATE ION IN THE REDUCTION OF COPPER SULPHATE

The structure of the tartrate or citrate complex has been discussed by Bobtelsky and Jordan[4] and these authors have given the following formulae for the copper complex.

1.

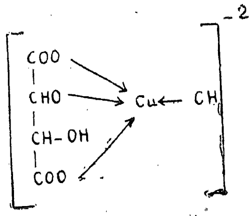


Copper-tartrate complex ... (A)



Copper citrate complex ... (B)

These complexes can react with sodium or potassium hydroxide. The hydrogen atom marked with an asterisk combines with hydroxyl of the base when excess of alkali is added. In case of the tartrate complex (and perhaps in case of citrate also) an additional equivalent of sodium hydroxide can be taken up and the following negative complex ion is formed.



Copper-tartrate complex ... (C).

Ultimately therefore the tartrate complex is able to use up two equivalents of alkali. It is necessary to emphasize this point here that the reaction starts when the negative complex (C) has been formed. The reaction does not occur at all even at the boiling point if the amount of alkali and the tartrate (or citrate) is less than represented by the following ratio :

$$\begin{array}{ccc} \text{Cu} & : \text{tartrate (or citrate)} : & \text{NaOH} \\ 1 : & 1 & : 2 \end{array}$$

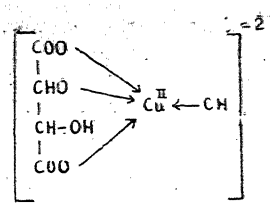
which corresponds to the formation of the negative complex which has taken up two equivalents of the base. In case of those experiments where citrate has been used the actual amount of citrate ion used is slightly more than the above ratio. This is because the citrate complex is less stable. The dissociation constant of the non-negative complex corresponding to the following reaction :

$$Cu^{++} + Cit'' \rightleftharpoons [Cu^{II}-Cit]$$

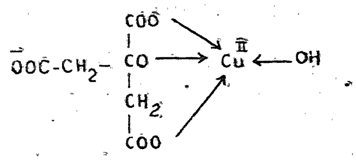
has been calculated by the above authors and the following table shows that less than 70% of the complex is formed at the 1 : 1 ratio of Cu : Cit.

$\dfrac{C_{Cu^{II}}}{C_{Cit^{II}}}$	% of complex	$K = \dfrac{C_{Cu^{II}} \times C_{Cit}}{C_{Cu^{II}-Cit}}$
0.855	70	1.0×10^{-3}
0.638	80	1.0×10^{-3}
0.195	95	1.1×10^{-3}

Bobtelsky and Jordan[4], however, have stated that while in case of the tartrate complexes (A) and (B) definitely exists, in case of citrate the complex corresponding to formula (C) does not exist. We are, however, of the opinion that in case of citrate also the complex corresponding to the formula (C) does exist, since the reaction does not start unless two equivalents of sodium hydroxide have been added. Hence we maintain that for the reduction of copper by Fehling's solution in presence of citrate or tartrate the Cu^{II} must exist in the following form;



Cu^{II} tartrate complex



Cu^{II} citrate complex

OXIDATION POTENTIAL OF CUPRO—CUPRIC SYSTEM

From oxidation potential measurements it is known that cuprous ion is unstable with respect to cupric ion as well as metallic copper. From the electrode potential of the following electrodes :—

$$Cu/Cu^{+},\ E_1 = -\ 0.520 \text{ Volts}$$

$$Cu/Cu^{++},\ E_2 = -\ 0.340 \text{ Volts}$$

one can calculate the equilibrium constant of the reaction $2Cu^{+} \rightleftharpoons Cu + Cu^{++}$

The K for the above reaction is found to be equal to 10^6 which shows that the reaction from left to right is practically complete. Hence a solution which contains cupric and cuprous ion cannot have more than $\frac{1}{50,000}$ of the copper present as cuprous ion. Reduction of cupric ion to cuprous can take place only when the resultant cuprous ion can be removed in the form of an insoluble compound for example as Cu_2O.

If we start with pure cupric sulphate, the cupric ion after reduction to cuprous may again be formed due to back reaction.

It appears that the function of citrate or the tartrate complex is first to stabilise the cuprous form of copper which is formed as a result of reduction of the bivalent copper citrate or the tartrate complex. The resulting cuprous citrate or the tartrate then gives rise to cuprous oxide.

No data are available regarding the stability constant of cuprous citrate or cuprous tartrate complexes, but it is known that the cuprous form is stabilised by the formation of various types of complexes, *e. g.* $CuCl_2^-$ is formed in presence of chloride ion (Cl^-) as shown by the following equilibrium constant :—

$$CuCl_2^- = Cu^+ + 2Cl^-,\ K = 2{\cdot}9 \times 10^{-6}$$

Similarly the cuprous form becomes stable in presence of cyanide ions as shown the following reaction $Cu(CN)_2^- = Cu^+ + 2\ CN^-,\ K = 10^{-16}$

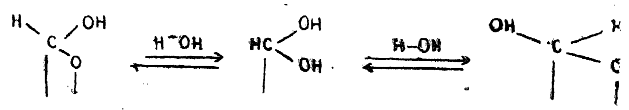
Similarly the function of tratrate and citrate complexes is to stablise the resulting cuprous form which facilitates the formation of insoluble cuprous oxide.

IS α,—β TRANSFORMATION OF SUGAR AS THE RATE DETERMINING STEP?

Some discussion regarding the mechanism of $\alpha - \beta$ transformation is being introduced here to counteract the possibility of the suggestion that in the oxidation

of sugar by Fehling's solution the intramolecular rearrangement that we have postulated in previous publications is no more than the transformation of α–dextrose to β – dextrose or vice-versa. In other words one might argue that the rate datermining step consists of solely of mutarotation of the sugar taken. This suggestion is absolutely incorrect as the following calculation will show :—

It is well known that the solution of reducing sugar in water (*e.g.* dextrose, fructose, etc.) exhibits the phenomenon of mutarotation which is subject to acid base catalysis. In α - β transformation the position of OH group and H atom is inverted in case of first carbon atom :—



α-hexose (cyclic form) | α-cyclic form of the hexoses | β-hexose (cyclic form)

The above scheme summarises the mutarotation of sugar.

Now the mutarotation is a reversible process as shown by the following scheme.

$$\alpha\text{ - form} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \beta\text{ - form}$$

and the measured velocity constant is given by the following equation :—

$$K\text{ (observed)} = K_1 + K_2 = \frac{1}{t} \log_e \frac{\gamma_0 - \gamma_\infty}{\gamma_t - \gamma_\infty}$$

where γ is the rotation shown by the sugar solution.

Further, Hudson gave the following equation for K (observed) at 25°C.

$$K\text{ (observed)} = 0.0096 + 0.258\, C_{H^+} + 9750\, C_{OH^-}$$

In a decinormal solution of NaOH ($C_{OH^-} = 10^{-1}$, $C_{H^+} = 10^{-13}$) the value of K (observed) will be :—

$$K\text{ (observed)} = 0.0096 + 0.258 \times 10^{-13} + 9750 \times 10^{-1}$$

$$= 975.0096 \text{ (neglecting } 0.258 \times 10^{-13})$$

therefore K (obs.) $= 976 = K_1 + K_2$ ... (1)

Further it was shown by Rüber[5] that an equilibrium mixture α - and β - form consists of 36% α - form and 64% of β - form in case of dextrose the equilibrium constant is

$$K_e = \frac{K_1}{K_2} = \frac{64}{36} = 1\cdot77 \text{ approximately}$$

i. e. $K_1 = 1\cdot77\, K_2$ ... (2)

substituting this in (1), we get.

$$K_2 + 1{\cdot}77\ K_2 = 975$$

$$K_2\ (1 + 1{\cdot}77) = 975$$

$$\text{hence}\quad K_2\ \frac{975}{277} = 319 \text{ approximately}$$

$$\text{and } K_1 = 655$$

In our case the unimolecular velocity constant for dextrose at a concentration of 0·73N alkali and temperature 40°C is equal to $9{\cdot}77 \times 10^{-3}$ minute^{-1}. Assuming a direct relationship between (this is practically correct for dextrose specially at such concentration) the concentration of alkali and the unimolecular velocity constant, the value of K (unimolecular) at decinormal concentration of alkali will be about $1{\cdot}34 \times 10^{-3}$. Taking the average temperature coefficient as 3·67 the velocity constant at 20°C would be $\frac{1{\cdot}34 \times 10^{-3}}{(3{\cdot}67)^2} = 995 \times 10^{-7}$. This value is more than a million times smaller than either for the transformation of α - to β - dextrose or of β to α - dextrose. Hence to conceive of $\alpha - \beta$ transformation as the rate determinning step is entirely absurd.

PREVIOUS TREATMENT OF SUGAR WITH ALKALI

It has been shown in the experimental part previously published that when glucose is kept for some time in alkali and then made to react with Fehling's solution, the velocity of reduction of Fehling's solution is increased, and longer the period during with glucose is kept in alkaline solution before it is oxidized, the greater is the velocity of reaction between Fehling's solution and glucose. This enhancement in velocity is due to the fact that a part of glucose is converted into fructose which has higher velocity of oxidation by alkaline copper sulphate solution.

On the other hand previous treatment of fructose with alkali leads to decrease in the reaction velocity. This is due to the fact that some glucose is produced due to intramolecular re-arrangement and it has a slow velocity of reduction. These facts point to the fact that perhaps the intermediate activated from is enediol form, which is formed during Lobry de Bruyn transformation[6]. A detailed discussion is now given below.

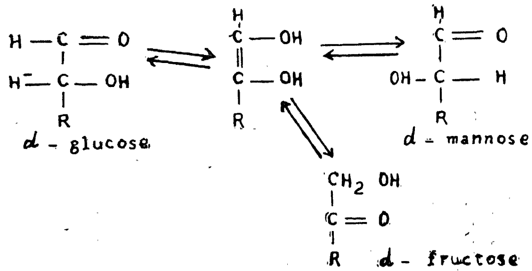
Now we take up the basic question of evaluating the exact nature of intramolecular rearrangement. The intramolecular rearrangement of sugar in alkaline medium was investigated by Lobry de Bruyn and Alberda Van Ekenstein[6] and later on was studied in great detail by Nef[7]. He pointed out that intermediate enediol is produced by the action of alkali on sugar molecule. On the basis of intermediate enediol we propose the following mechanism in the oxidation of reducing sugar by Fehling's solution :—

$$\text{Reducing sugar} \xrightarrow[\text{Catalysed by OH ion (slow step) fast}]{\text{rate determining step}} \text{active enediol} \quad \ldots \quad (a)$$

$$\underset{\text{(or tartrate) complex}}{\text{Active enediol + Copper-citrate}} \longrightarrow Cu_2O + R - COOH + H_2O + CO_2 \quad (b)$$

Steps (a) and (b) are quite adequate to explain the zero molecularity of copper citrate or copper tartrate complex and the unimolecularity of the sugar taken. Step (a) is catalysed by the OH ions while step (b) is a very fast reaction.

More exact work has been done on the interconversion of dextrose and fructose by Morrel and Bellars[8] and by Wolfrom and Lewis[9]. The latter authors have studied the following equilibrium :—



The composition of the mixture formed by the action of calcium hydroxide on *d*-glucose was shown as follows :—

glucose	... 69·4%	fructose	... 30·9%
mannose	... 2·4%	non-sugar substances (saccharic acid)	... 3·3%

The above equilibrium was attained in about 270 hrs., and a recalculation of their data shows that the reaction is not exactly unimolecular. These authors themselves have not ascribed any order to the reaction. The presence of acids in the equilibrium mixture must be ascribed to the action of atomspheric oxygen (*See* Gilman: Organic Chemistry Vol. II, p. 1645). For simplicity we shall neglect mannose and consider the equilibrium between *d*-glucose and *d*-fructose :—

$$d\text{-glucose} \leftarrow d\text{-fructose}$$

$$69\% \underset{K_2}{\overset{K_1}{\rightleftarrows}} \leftarrow 31\% \qquad \ldots (a)$$

Further we may assume that the reaction behaves unimolecularly on either side. Since the composition of equilibrium mixture is known, we can easily calculate the ratio of K_1 to K_2, We have therefore

$$\frac{K_2}{K_1} = \frac{69}{31}\ 2\cdot22 \text{ approximately} \qquad \ldots (b)$$

A more systematic work from kinetic point of view was carried out by Morrel and Bellars in 1907 on conversion of dextrose guanidine into fructose guanidine. They have shown that guanidine is similar in its catalytic action to sodium or potassium hydroxide and on the basis of this assumption, they have identified the following equilibrium:

$$d\text{-glucose guanidine} \underset{K^1_2}{\overset{K^1_1}{\rightleftharpoons}} d\text{-fructose guanidine} \qquad \ldots (c)$$

with the equilibrium:

$$d\text{-glucose} \rightleftharpoons d\text{-fructose} \qquad \ldots \qquad (d)$$

on this basis $K^1_1 = K_1$ of eqn. (*a*), and $K^1_2 = K_2$. The values of K^1_1 and K^1_2 respectively were shown to be equal to 0·0015 minutes^{-1} and 0·0021 minutes.$^{-1}$

$$\text{Hence } \frac{K^1_2}{K^1_1} = \frac{K_2}{K_1} = \frac{0{\cdot}0021}{0{\cdot}0015} = 1{\cdot}47 \text{ approx.}$$

It may be pointed out here that in case of Lewis and Wolfrom the unit of time was hour while in case of Morrel and Bellars the unit was minute. But for the present the unit does not matter since we are dealing with the ratio of velocity constants. Wolfrom and Lewis carried out the experiment in presence of lime and at pH 10·6, at higher pH the velocity constants would have certainly increased. In case of Morrells and Bellars, work the higher value for velocity constant is due to presence of guanidine which is a strong base.

In order to find out which of equilibrium ratios, i.e. as given by Wolfrom and Lewis or that by Morrel and Bellars is more accurate, we may add that Wolfrom and Lewis worked with pure dextrose and hence their value must be taken to be more accurate, although it must be pointed out that the work of Morrel and Bellars is more neat and commendable from the kinetic point of view. Morrel and Bellars have hardly received justice at the hands of organic chemists who have rarely referred to their work.

We therefore assume that the equilibrium ratio presented by Lewis and Wolfrom is correct one *i. e.*

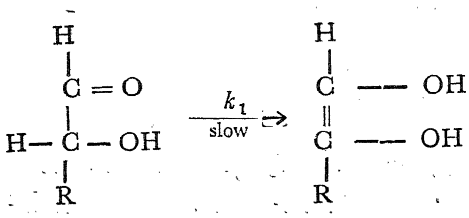
$$\frac{K_2}{K_1} = 2{\cdot}22 \qquad \ldots \qquad (1)$$

The equilibrium (*a*), (*c*) or (*d*) omits the enediol form from consideration, but from our point of view the intermediate enediol form is of fundamental importance. The actual amount of the enediol form is so small that we may introduce it in the above equilibrium without disturbing the percentage of d-glucose or d-fructose.

The entire equilibrium between d-glucose and d-fructose (neglecting mannose) including enediol form may be presented as follows:

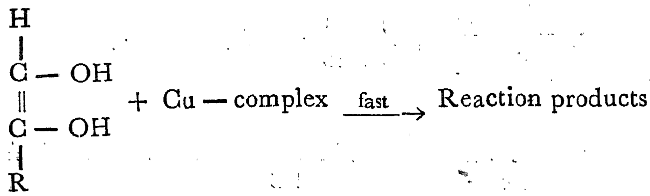
$$d\text{-glucose} \quad \underset{\xleftarrow[\quad K_2 \quad]{}}{\overset{\xrightarrow{\quad K_1 \quad}}{\underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{enediol form} \underset{k_4}{\overset{k_3}{\rightleftharpoons}}}} \quad d\text{-fructose} \qquad \ldots \qquad (f)$$

Taking the *k's* into consideration we may now present the mechanism of reaction say between d-glucose and Cu-complex as follows:



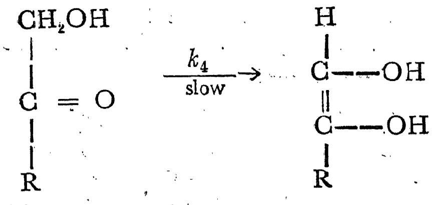
... 1st step

d-glucose (1,2 enediol)



...2nd step

With d-fructose the 1st step is prsented as follows :—

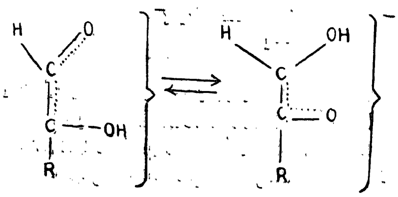


... 1st step

d-fructose (1,2 enediol)

The second step will be exactly as in case of *d*-glucose.

Recently Bamford and Collins[10] have studied the oxidation of glucose and fructose by oxygen in strongly alkaline medium and they are of the opinion that in the inter conversion of glucose and fructose the following two enolate ions exist which are considered to be in tautomeric equilibrium.



Whatever may be the intermediate, *i.e.* either enediol or enolate ion, but our main point is the rate of oxidation of aldehydo hexose or pentose by Fehling's solution represents the rate of enolization of the aldehydo sugar while the rate of oxidation of keto sugar represents the rate of enolization of the keto sugar.

In both cases the double bond enters between the first and second carbon atoms. There is of course no direct proof for this kind of contention for separate values for k_1, k_2, k_3 and k_4 are not known. Indeed it is almost impossible to measure them separately. The only directly measured values in the equilibrium (f) are those K_1 and K_2 as given by Morrel and Bellars, or the values for the ratio $\frac{K_2}{K_1}$ as calculated by us from the data of Wolfrom and Lewis.

Let us again consider the equilibrium (f)

Let g=concentration of d-glucose

f=concentration of fructose

e=concentration of enediol

Then $\frac{e}{g}=\frac{k_1}{k_2}$, and $\frac{f}{e}=\frac{k_3}{k_4}$ ... (2)

and $\frac{e}{g}\times\frac{f}{e}=\frac{k_1\times k_3}{k_2\times k_4}=\frac{K_1}{K_2}=\frac{f}{g}$ ... (3)

Now from eq. (1)

$$\frac{g}{f}=\frac{K_2}{K_1}=2{\cdot}22=\frac{k_4\times k_2}{k_1\times k_3} \quad \text{... (4)}$$

$$\text{or } 2{\cdot}22\times\frac{k_3}{k_2}=\frac{k_4}{k_1} \quad \text{... (5)}$$

The ratio between the unimolecular velocity constants of oxidation for d-fructose and d-glucose under identical conditions, which we have measured, is 2·30. If the mechanism of oxidation of d-glucose and d-fructose were correct then,

$$2{\cdot}3=\frac{k_4}{k_1}=2{\cdot}22\frac{k_3}{k_2} \quad \text{... (6)}$$

But the above contention cannot be valid, unless it can be proved that the $\frac{k_3}{k_2}$ approximates to unity. There is no direct evidence that $\frac{k_3}{k_2}$ is 2·22 times less than the ratio $\frac{k_4}{k_1}$. Further we assume, in absence of evidence to the contrary that $\frac{k_3}{k_2}$ approximate to unity. At any rate this cannot be disproved from the present data. We, therefore, arrive at the following fundamental point :—

The rate of oxidation of a hexose or pentose sugar by Fehling's solution is equal to the rate of enolization or enediol formation at the 1,2 carbon atoms.

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ON SOME THEOREMS ON POISSON DISTRIBUTION

By

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INTRODUCTION

Sec. I.—The Poisson's distribution will be applied in Applied Science.

Sec. II.—Wisharat (1949) has found the cumulants of Multivariate Multinominal distribution. In this section Moments and Cumnlants of Multivariate Poisson's distribution will be found.

Sec. I

Theorem.—If the X rays are regarded as discrete quanta and appear independently and at random then the probability, that in t seconds x of the X rays hit the organism of size S whose projection on a plane is an area a, is $P(x, t) = \frac{e^{-m} m^x}{Lx}$ where m is the average number of rays striking an area of size a in t seconds.

Now $$P(x, t+dt) = P(x, t)\, P(o, dt) + P(x-1, t)\, P(1, dt) + \ldots + P(o, t)\, P(x, dt)$$

Again $$P(o, dt) = 1 - P(1, dt) - P(2, dt) \ldots \ldots \ldots$$

Hence $$\frac{P(x, t+dt) - P(x,t)}{dt} = [P(x-1, t) - P(x, t)] \times \frac{P(1, dt)}{dt} + [P(x-2, t) - P(x, t)]\frac{P(2, dt)}{dt} + \ldots \ldots \ldots$$

Following Fry (1928) we can prove that

$$\frac{[P(1, dt)]}{dt} \to K(t) \text{ as } dt \to 0 \text{ and } \frac{[P(2, dt)]}{dt} =$$

$$\frac{[P(3, dt)]}{dt} = \ldots = 0 \text{ as } dt \to 0$$

Hence $$\frac{d\,P(x, t)}{dt} = K(t)\,[P(x-1, t) - P(x, t)]$$

Since the number of rays cannot be negative we have

$$\frac{dP(o, t)}{dt} = -K(t)\, P(o, t).$$

Hence $P(o, t) = Co\, e^{-k_1(t)}$ where $K_1(t) = \int_0^t K(t)\, dt.$

Since $P(o, o) = 1,\ Co = 1$

Hence $P(o, t) = e^{-k_1(t)}$

$$\frac{dP(1, t)}{dt} = K(t)\,[P(o, t) - P(1, t)]$$

Hence $$\frac{dP(1, t)}{dt} + K(t)\, P(1, t) = K(t)\, e^{-k_1(t)}$$

Hence $$P(1, t) = G\, e^{-k_1(t)} + e^{-k_1(t)} \int_0^t e^{k_1(t)}\, P(o, t)\, K(t)\, dt$$

$$= C\, e^{-k_1(t)} + e^{-k_1(1)} \int_0^t K(t)\, dt$$

$$= G\, e^{-k_1(t)} + e^{-k_1(t)}\, K_1(t)$$

Since $K_1(0) = 0$ and $P(1, 0) = 0$

We have $G = 0$

Hence $P(1, t) = e^{-k_1(t)}\, K_1(t)$

Proceeding in this way have $P(x, t) = e^{-k_1(t)} \dfrac{k_1(t)^x}{Lx}$

Again $$E(x) = \sum_{x=0}^{a} x\, P(x, t) = K_1(t) = m$$

Hence $$P(x, t) = \frac{e^{-m}\, m^x}{Lx}$$

This result has been assumed by Wilks (1943) without proof.

Corollary.—If the absorption of K or more of the X rays kill a certain unicelluler organism then the average number of rays absorbed by the survivors is

$$\frac{\sum_{x=0}^{k-1} \dfrac{x\, m^x}{Lx}}{\sum_{x=0}^{k-1} \dfrac{m^x}{Lx}}$$

Where m is the average number of X rays striking an area a in t seconds.

Since the probability of survival is $\sum_{x=0}^{k-1} \dfrac{e^{-m}\, m^x}{Lx}$ we have the result.

Sec. II

Let $p_1, p_2 \ldots p_n$ be the probability of happening of a rare event in any one of n ways in a random sample of s trials. Hence the probability of x_1 successes of

the 1st kind, x_2 of the second kind......and x_n of n the kind is

$$\frac{Ls}{Lx_0\ Lx_1\ \dots\ Lx_n}\ p_0^{x_0}\ p_1^{x_1} \dots p_n^{x_n}$$

Where $p_0 = 1 - \sum_1^n p_i$

and $x_0 = S - \sum_1^n x_i$

The probability generating function $\theta\ (t_1\ t_2 \dots t_n)$

$$= [p_0 + p_1 e^{t_1} \dots + p_n e^{t_n}]^s = \left[1 + \sum_{i=i}^{n} \frac{\lambda_i\,(e^{t_i} - 1)}{s}\right]^s$$

if $\quad p_1 = \frac{\lambda_1}{s},\ p_2 = \frac{\lambda_2}{s}\ \dots\ p_n = \frac{\lambda_n}{s}$

$$\theta\ (t_1\ t_2\ \dots\ t_n) = e^{\sum_{i=1}^{n} \lambda_i\,(e^{t_i} - 1)} \text{ when } S \to \infty$$

$p_1, p_2 \cdot\cdot p_n \to o$

Now $\quad \frac{\partial\theta}{\partial t_i} = \theta\ \lambda_i\ e^{t_i}$

and $\quad \lambda_i \frac{\partial\theta}{\partial\lambda_i} = \theta\ \lambda_i\ (e^{t_i} - 1)$

Hence $\quad \frac{\partial\theta}{\partial t_i} = \lambda_i \frac{\partial\theta}{\partial\lambda_i} + \lambda_i\,\theta$

$$\mu^1 \dots\dots 1 = \left(\frac{\partial\theta}{\partial t_i}\right)_{\substack{t_i = 0\\ tj = 0}} i \neq j$$
$$= \lambda_i$$

We have the moments upto third order.

$\mu^1 \dots 1 \dots = \lambda_i,\ \mu^1 \dots 2 \dots = \lambda_i\,(1 + \lambda_i),\ \mu^1 \dots 11 \dots \lambda_i\,\lambda_j,$

$\mu^1 \dots 21 \dots = \lambda_i\,\lambda_j\,(1 + \lambda_j),\ \mu^1 \dots 111 \dots = \lambda_i\,\lambda_j\,\lambda_k$

$$\mu^1 r_1 \dots r_{i+1} \dots r_n = [\lambda_i \frac{\partial}{\partial\lambda_i} + \lambda_i]\ \mu^1\ r_1\ r_2 \dots r_n$$

$$\mu^1_{r+1} = \lambda_r \frac{\partial\mu^1_r}{\partial\lambda_r} + \lambda_r\,\mu^1_r\ (r \geqslant 1)$$

$$\mu^1_{r+1\ r_2+1 \dots r_n+1} = \prod_{i=1}^{n} \left[\lambda_i \frac{\partial}{\partial\lambda_i} + \lambda_i\right]^{r_i} \lambda_1 \cdot \lambda_2 \dots \lambda_n$$

Again $\mu^1 ... 2 ... = \left(\frac{\partial^2\theta}{\partial t_1^2}\right) t_1 = 0 = \lambda_i + \lambda_i^2 = \mu^1 ... 1 + \mu^{12} ... 1 ...$
$= t_j$

$$\mu^1 ... 3 = \left(\lambda_i \frac{\partial}{\partial\lambda_i} + \lambda_i\right) \mu^1 ... 2 ... = \lambda_i\,(1 + 3\lambda_i + \lambda_i^2)$$

$$= \mu^1 ... 1 ... + 3\mu^{12} ... 1 + \mu^{13} ... 1 ...$$

If μ_r be the moment about the mean the moment generating function

$$\theta^1\,(t_1\, t_2 ... t_n) = e^{-(\lambda_1 t_1 + ... + \lambda_n t_n)}$$

$$= e^{\Sigma\, \lambda_i\,(e^{t_i} - 1 - t_i)}\ \theta\ (t_1 \cdot, \cdot t_n)$$

$$\frac{\partial\theta^1}{\partial t_i} = \theta^1 \lambda_i\,(e^{t_i} - 1), \quad \frac{\partial\theta^1}{\partial\lambda_i} = \theta^1\,(e^{t_i} - 1 - t_i)$$

Hence $\frac{\partial\theta^1}{\partial t_i} = \lambda_i \left(\frac{\partial}{\partial\lambda_i} + t_i\right)\theta^1$

$$\frac{\partial^{r+1}\,\theta^1}{\partial t_i^{r+1}} = \lambda_i \left[\frac{\partial^r}{\partial\lambda_i^r}\ \frac{\partial^r\,\theta^1}{\theta t_i} + t_i\,\frac{\partial^r\,\theta^1}{\partial t_i^r} + \frac{r\partial^{r-1}\,\theta^1}{\partial t_i^{r-1}}\right]$$

Hence $\mu_r + 1 = \lambda_i \frac{\partial\mu_r}{\partial\lambda_i} + r\lambda_i\,\mu_{r-1}$

$$\mu_{r_1} + 1\ r_2 + 1 .. r_n + 1 = \lambda_1\,\lambda_2 ... \lambda_n\ \frac{\partial^n\,\mu\, r_1 ... r_n}{\partial\lambda_1\,\partial\lambda_2 ... \partial\lambda_n}$$
$$+ \lambda_1\,\lambda_2 \cdots \lambda_n\, r_1\, r_2 \cdots r_n\, \mu_{r_1} - 1\, r_2 - 1 ... r_n - 1$$

Let $K(t)$ be the Cumulant generating function

Then $K(t) = \Sigma\,\lambda_i\,(e^{t_i} - 1)$

$$\frac{\partial k}{\partial t_i} = \lambda_i\,\frac{\partial k}{\partial\lambda_i} + \lambda_i = K.\,.1.\,. + \lambda_i\,\frac{\partial k}{\partial\lambda_i}$$

Hence $K r_1\, r_2 ... r_1 + 1 ... r_n = \lambda_i \frac{\partial}{\partial\lambda_i}\ (K r_1\, r_2 ... r_n)$

$$K r + 1 = \lambda\,\frac{\partial k r}{\lambda}\ (r \geqslant 1)$$

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CHEMICAL EXAMINATION OF THE SEEDS OF *PHYLLANTHUS MEDARASPATENSIS LINN* : THE STUDY ON MUCILAGE

By

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Chemical examination of the seeds of Phyllanthus medaraspatensis (HINDI-Kanocha) showed the presence of an oil, mucilage, colouring matter and essential oil.

The mucilage, isolated in chemically pure form, on chemical examination showed that it belonged to the polyuronide group of natural organic compounds. The complex molecule of it was made up of sugars and sugar acids.

The mucilage was extracted from oil free seeds. The freshly extracted mucilage was dirty white in colour and fibrous in nature. On long exposure to air it turned brown. It was insoluble, almost, in all the organic solvents such as, petroleum ether, ether, benzene, chloroform, acetone, alcohols and ethyl acetate. Its solubility in distilled water was very low (refractive index of the solution of mucilage was found to be 1·3331, while that of the distilled water was found to be 1·3336).

The viscosity of the saturated solution of the mucilage was found to be 1·79 (pH 2·5) at 20·5°C and 20 m. m. pressure. The equivalent weight of the mucilage was found to be 1090 approximately. Complete incineration of the pure mucilage gave 4·6% of a greyish white ash, the qualitative analysis of which revealed the presence of calcium, iron and magnesium. The quantitative estimation of the ash gave the following results:

Calcium = 1·2%; Iron - 1·0% and Magnesium - 1·1%.

The ultimate analysis of the pure mucilage gave C=37·31%; H=7·1%.

On estimation, methoxy and acetyl groups were found to be absent in the molecule.

The graded acid hydrolysis was carried out by treating the mucilage with 3% oxalic acid; 2-N sulphuric acid and conc. sulphuric acid successively.

The 3% oxalic acid hydrolysis gave galactose and galactouronic acid; 2N-sulphuric acid gave arabinose, galactose, rhamnose and aldobionic acid. The conc. sulphuric acid hydrolysis of the residue, left after separation of the water soluble products of 3% oxalic acid and 2-N sulphuric acid hydrolysis gave only glucose which showed that the residue was made up of cellulose molecules only.

In the hydrolysis experiments, total percentage of reducing sugars was found to be 14·2% in oxalic acid; 24·29% in 2-N sulphuric acid and 20·28% in the conc. sulphuric acid hydrolysis.

The oxalic acid hydrolysis started after a lapse of six and a half hours and in case of 2-N sulphuric acid after a lapse of five to six hours. From these observations it was inferred that some sort of distintegration had taken place during the hydrolysis.

Chromatographic studies, of the test portions of the sugars obtained, as a product of graded acid hydrolysis at intervals showed that the intensity of the spot for galactose decreased, on proceeding from oxalic acid to 2-N sulphuric acid hydrolysis. The intensities of the spots indicating the sugars obtained from 2-N sulphuric acid hydrolysis decreased towards the completion of the hydrolysis.

EXPERIMENTAL

Extraction of the Mucilage—Six hundred gms. of the powdered seeds from which the oil and essential oil were exhaustively extracted before hand with petroleum ether (b. p. 40° - 60°C) and steam distillation respectively were soaked in distilled water (6l) acidified with acetic acid (30 c.c.). The contents of the container were then stirred vigorously with a mechanical stirrer. After 25 to 26 hours the contents of the container were filtered through a muslin cloth. The acidic filtrate was added to large volume of rectified spirit and stirred when whole of the fibrous mass was precipitated out. This mass was then separated out by centrifugal method. (Yield=91·62 gms; 15·27%).

Purification—The crude mucilage (80 gms.) was first dissolved in hot distilled water (5l) acidified by acetic acid (30 c.c.) and stirred vigorously. It was then filtered. The filtrate was added to absolute alcohol and stirred when the mucilage was completely precipitated out. The precipitate was then taken out and dried. The dried mucilage was then refluxed successively with petroleum ether, ether, benzene, chloroform and alcohol (to remove all other organic substances which might be contaminated with it). Finally, the pure mucilage was dried under vacuum.

The ultimate analysis of the pure mucilage gave (C=37·31% and H=7·1%).

The percentage of the ash was found to be 4·6%. The qualitative chemical analysis of the ash, revealed the presence of calcium, iron and magnesium. The results of gravimetric estimation of the ash are given below:

Found: Calcium=1·2%; Iron=1·0% and Magnesium=1·1%

Equivalent Weight—A weighed amount of mucilage was refluxed for two and a half hours, with a known volume of standard N/10 caustic soda solution. The solution was cooled and the excess of alkali was titrated against the standard solution of N/10 hydrochloric acid. From this the equivalent weight of the mucilage was calculated which was found to be 1090 approximately.

HYDROLYSIS OF THE MUCILAGE

1. With Oxalic Acid: 40·0 gms. of the pure mucilage was taken, in a pyrex beaker, to it was added 10 gms. of oxalic acid in 400 c. c. distilled water. It was then heated on a water bath and stirred at intervals.

After starting the experiment, a test portion was taken out at intervals from the reaction mixture and each time it was tested with Fehling's solution. After

six and a half hours the test portion was found to reduce the Fehling's solution indicating, thereby, that the hydrolysis had started. The heating was continued until the test porition from the reaction mixture reduced the Fehling's solution. The test portions ceased to reduce the Fehling's solution after 26 hours and the heating was stopped.

Isolation of the products of Hydrolysis:—The contents of the beaker were filtered through a sintered funnel. The residue on the funnel was kept for subsequent hydrolysis. To the filtrate an excess of barium carbonate was added (to precipitate the excess of oxalic acid, as barium oxalate and further to convert the sugar acids into their soluble barium salts). After that the precipitated barium oxalate and excess of barium carbonate was filtered. The filtrate contained the sugars and the barium salt of the sugar acids.

Separation of the Sugars and Sugar Acids:—The above filtrate was concentrated to a syrup (under reduced pressure 20 m. m.) on a water bath. To this syrup excess of methyl alcohol was added (The methanol precipitated the barium salt of the sugar acids while the sugars remained in solution).

The precipitate (which is the barium salt of the sugar acids) was separated out by filtration, washed well with methanol and finally dried (under vacuum) and kept for the identification of sugar acids. From the filtrate the methanol was distilled off and the syrup thus left was kept for the identification of the sugars. Thus the products of hydrolysis were divided into (A) sugars and (B) sugar acid portions. These two portions were analysed as follows:

(A) *Sugars:*—The syrup of sugars was taken in 50 c. c. of distilled water. It was then purified by treating with animal charcoal and desalting. The pure sugar mixture was then subjected to chromatographic examination.[1]

The chromatographic study showed the presence of only one indicative spot which corresponded to galactose.

Confirmation:—The indicated galactose was confirmed as follows:

(*i*) O-Toly-hydrazone derivative: 20 c. c. of the hot concentrated aqueous solution of the sugars spot indicating galactose were heated in 20 parts alcohol with a solution of 1% tolyl phenylhydrazine for 30 mts. On cooling colourless needles separated out. These on further recrystallization from alcohol melted at 175-76°C which corresponded to galactose O-tolyl-hydrazone.

(*ii*) Oxidation of the Sugar: 10 c. c. of the sugar solution was heated with conc. nitric acid on a water for 1 to 2 hours, when a white acidic product was obtained, which on further recrystallization with alcohol melted at 210-11°C and was identified to be mucic acid.

Thus the presence of galactose was confirmed in the hydrolysis.

Total percentage of reducing sugars:—To estimate the total percentage of reducing sugars in the hydrolysis, a known volume of the hydrolysed solution was titrated against a standard Fehling's solution using methylene blue an indicator. From it the percentage of the sugars in the hydrolysis was calculated.

Found; Total percentage of reducing sugars = 14·2%

(B) *Sugar Acid*:—To identify the sugar acid barium in the barium salt, obtained during the separation of the sugar acid was estimated as barium sulphate and it was found to correspond with the percentage of barium in the barium salt of galacto-uronic acid.

Found : Barium=26·82 % ; Calculated value for the galacto-uronic Acid= 26·9%

Thus the sugar acid was identified to be galactouronic acid.

The above study of the oxalic acid hydrolysis of the mucilage revealed the presence of galactose and galactouronic acid in the molecule of the mucilage.

2. *With 2-N Sulphuric Acid* :—The residue of the mucilage, left after separating the water soluble products of oxalic acid hydrolysis was taken into a pyrex beaker containing 60 c. c. of 2-N sulphuric acid. It was then heated on a water bath.

The test portions taken from the reaction mixture in the case of oxalic acid hydrolysis showed that the hydrolysis started after a lapse of five to six hours and the reaction took 26 to 27 hours for completion. After that the contents of the beaker were filtered and the residue was kept for concentrated sulphuric acid treatment. From the filtrate the products of hydrolysis were isolated, separated and purified in the same way as the products of oxalic acid hydrolysis. On separation the products of hydrolysis were found to be (*a*) Sugars and (*b*) Sugar acids. These were examined as given below :

(A) *Sugars*:—The pure mixture of sugars on chromatographic examination[1] revealed the presence of galactose, arabinose and rhamnose.

Confirmation :—To confirm these sugars their derivatives with phenylhydrazine hydrochloride were prepared by taking a hot concetrated aqueous elution of each indicative spot. The products obtained on further recrystallization with alcohol corresponded to galactose, arabinose and rhamnose phenylhydrazones respectively.

The total percentage of reducing sugars :—It was estimated by the same method as was followed in the case of oxalic acid hydrolysis.

Found : Total percentage of reducing sugars-24·29%

(B) *Sugars Acid* :—The barium in the barium salt of the sugar acid was estimated which corresponded to the percentage of barium in the aldobionic acid.

Found : Barium 16·42%, Calculated value for the aldobionic acid—16·18%.

Confirmation :—The barium salt was oxidised by conc. nitric acid. The oxidation product was identified to be mucic acid (m.p. 210-11°C).

3. *With Conc. Sulphuric Acid* :—The residue of the mucilage, left after separating the water soluble products of 3% oxalic acid and 2-N sulphuric acid hydrolysis of the mucilage was finally hydrolysed by conc sulphuric acid hydrolysis.

The separation of the products with barium carbonate showed the absence of sugar acid portion. The sugar portion was examined as follows :—

(A) *Sugar* :—The chromatographic study[1] of the sugar solution revealed the presence of only one indicative spot which corresponded to glucose.

Confirmation :—The glucose was confirmed by preparing the osazone derivative of the sugar spot corresponding to glucose. The osazone thus obtained, on purification with alcohol melted at 204-05°C and corresponded to glucose phenylosazone.

Total percentage of glucose :—It was estimated by the same method as in case of oxalic acid hydrolysis of the mucilage.

Found :—Percentage of glucose=20·28%

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KINETICS OF REDUCTION OF ALKALINE BIVALENT COPPER BY REDUCING SUGARS

PART IX. THE CATALYTIC ACTIVITY OF CUPROUS OXIDE

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ABSTRACT

The autocatalysis due to cuprous oxide has been discussed in this paper.

The common method for the estimation of a reducing sugar consists in oxidising the latter by bivalent copper in the form of Fehling's or Benedict's solution.[1] This may be effected directly by titrating a standard Fehling's or Benedict's solution against the sugar solution in presence of methylene blue as indicator, or as an alternative by estimating the resulting cuprous oxide[2] (Bertrand method)[3] by dissolving the latter in acidic pure ferric sulphate solution and titrating the ferrous formed equivalent to cuprous oxide by a standard oxidising agent e.g. potassium dichromate, potassium permangnate or ceric sulphate. The over all reaction between bivalent copper and a reducing sugar, say dextrose or fructose may be represented by the following scheme :—

$$R.\ CH(OH).\ CHO + 6CuO \rightarrow R.\ COOH + H_2O + CO_2 + 3Cu_2O$$

$$R.\ CO.CH_2OH + 6\ CuO \rightarrow R.\ COOH + H_2O + CO_2 + 3Cu_2O$$

In order to elucidate the structure of milk sugar and invert milk sugar, and the action of alkalies on reducing power (Reduction-Vermogen) of sugars in general, Urech[4] carried out a series of experiments including kinetical study of oxidation of dextrose by Fehling's solution. The kinetical measurements were made usually at temperatures below 20°C, and as is to be expected the reaction velocity was very slow. In the initial stages of the reaction, time could be measured in hours (instead of minutes as in our case) while in the later part of the reaction sometimes several days elapsed between any two consecutive readings. In some cases the entire reaction period extended to more than 20 days.

In the light of well known action of alkali on monosaccharides studied by several workers[5] and the powerful catalytic activity of cuprous oxide depending as it does upon the rate of dispersion of cuprous oxide and the size of the particles one may cast doubt upon the seriousness with which we have to study the results obtained by Urech as far as they relate to pure kinetics as we understand it in the modern sense. Indeed he himself was more concerned with studying the action of alkali on the reducing power and correctly arrived at the result, *viz.* the action of alkali in general leads to a decrease in the reducing value of sugars.

Urech tried to work out various formulae for velocity equation and finally obtained the following integral equation.

$$K(t+d) = \log Uo - \log U \qquad \dots (1)$$

where U is the concentration of glucose taken at time t and d is constant numerically equal to 6. It is obvious that equation (1) represents a first order kinetics with a slight modification as shown below.

By arranging the terms in equation (1) and putting it in the exponential form one can write equation (1) in the form

$$U = Ae^{-kt}, \qquad \text{where } A = U_0 e^{-kd} \qquad \dots (2)$$

Urech himself was unable to explain increase in velocity with time and introduced a term d to take care of experimental results. However from equation (1) and (2) it is clear that there is nothing which should show any increase in the velocity of reaction. The actual examination of the table in the paper[4] already referred to, shows that the value of K obtained was not constant. There was always a rapid increase in the value of K in the beginning upto 60 hours of the reaction. Urech however, was successful in showing that the formula (1) gave better results when the constant K was compared at different dilutions of normal Fehling's solution than the constant obtained by previous formula ($KV^y = Ut^{-x}$) when the constants were compared at different dilutions.

URECH'S FAILURE

Urech failed to formulate the exact mechanism of oxidation of dextrose by Fehling's solution since the modern concepts of reaction kinetics had not been properly developed at that time. Although Arrhenius' idea of activated molecule had been just put forward by that time, yet the concept of intermediate state as pre-requisite to the formation of reaction product had yet to be driven home. Urech failed to appreciate that the reaction product Cu_2O was a powerful catalyst and the increase in reaction velocity with time observed was due to the catalytic surface provided by the particles of Cu_2O. He could not make a proper choice of concentrations which resulted in his failure to understand the basic fact that the reaction was really independent of concentration of copper sulphate, nor did he make any attempt to understand the order of the reaction with respect to each reactant separately.

It is well known that if dextrose is kept for long time in the alkaline solution it is converted into laevulose and mannose[6] and at a higher concentrations of alkali it is decomposed[7] into such reaction products, which no longer can reduce Fehling's solution. Therefore, except for the initial period of reaction the results obtained by Urech cannot be taken seriously in so far as the oxidation of dextrose by Fehling's solution itself is concerned.

THE VELOCITY EQUATION

In previous publications[8,9,10,11] we have determined to total order of the reaction beetween reducing sugar and alkaline bivalent copper. It is directly proportional to sugar and independent of copper sulphate concentration. We also observed that reaction is catalysed by hydroxyl ions. The velocity equation can be exactly represented if we omit cuprous oxide catalysis from consideration, by the following equation :

$$-\frac{dU}{dt} = KU \qquad \dots (3)$$

where U is the concentration of the sugar taken at any time t. Owing to the powerful catalytic role of cuprous oxide, however, the exact order of the reaction is masked. It is only in presence of large excess of $CuSO_4$ concentration and small amount of sugar, that the exact unimolecular role of sugar becomes obvious. In such cases the amount of Cu_2O present in the peptised condition is very small and autocatalysis is restricted to the induction period only, and the reaction behaves as a first order reaction.

The zero molecular role of copper sulphate is a little less obvious. When the reaction is carried out in presence of large excess of the reducing sugar, no doubt, in certain cases, the reaction behaves, after a slight induction period, as a zero order reaction only. But in most cases a slight autocatalysis is present throughout the entire course of zero order reaction. This is, of course, due to Cu_2O produced which forms a colloidal solution in presence of large excess of sugar. If we omit the cuprous oxide catalysis, the reaction velocity in presence of large excess of the reducing sugar, becomes constant *i.e.*,

$$\frac{dx}{dt} = \frac{x}{t} = \text{constant} \qquad \dots (4)$$

as that the total order is again given by equation (3)

If we take catalysis of cuprous oxide into consideration and assume that velocity is directly proportional to the amount of Cu_2O produced, then equation (3) changes to:—

$$\frac{dx}{dt} = K(d - x)x \qquad \dots (5)$$

while eq. (4) changes to

$$\frac{dx}{dt} = Kx \qquad \dots (6)$$

We will consider equation (6) only since autocatalysis is present throughout the entire course of the reaction only in case of zero order constants. The integral form of eq. (6) can be written as

$$K = \frac{1}{T_2 - T_1} \log_e \frac{x_2}{x_1} \qquad \dots (7)$$

The application of this equation to a typical zero order table gives the following values for

T_2 (minutes)	T_1 (minutes)	X_2	X_1	$K = \frac{2 \cdot 303}{T_2 - T_1} \log_{10} \frac{x_2}{x_2}$
25	10	1·76	0·34	0·04769 × 2·303
40	10	3·00	0·34	0·03452 × 2·303
55	10	4·82	0·34	0·02560 × 2·303
60	10	5·33	0·34	0·02390 × 2·303
65	10	6·04	0·34	0·01922 × 2·303
70	10	6·82	0·34	0·01860 × 2·303
75	10	7·78	0·34	0·01913 × 2·303
80	10	9·14	0·34	0·01787 × 2·303

The above table shows at first a rapid fall in K and with the increase in t the K assumes a constant value.

In eq. (6) or (7) we have assumed that catalysis is directly proportional to the concentration of Cu_2O present at any time. This assumption is certainly not correct. If we go a little deeper into the theory of heterogeneous catalysis we have to consider the actual area of surface which is provided by entire amount of Cu_2O present at any instant. The actual calculation of the surface of contact is really a very difficult problem, since the extent of surface depends upon the total number of particles and their size at any instant. But these quantities are in a constant flux.

ADDITIONAL FACTORS THAT AFFECT AUTOCATALYSIS

Since the oxidation of hexoses or pentoses is heterogeneously catalysed by Cu_2O, all those influences that affect the state of dispersion or Cu_2O will influence the velocity of the reaction. The most powerful of all factors is the presence of large excess of the reducing sugar the Cu_2O is precipitated in a state of fine dispersion and usually a continuous autocatalysis is observed in such cases.

Sometimes the extent of autocatalysis may depend upon the actual magnitude of the velocity itself. The velocity of the reaction may be so fast that more and more of the primary particles are formed and their rate of disappearance may be more than balanced by the rate of appearance of the primary particles. In such cases the nature of the reaction will be highly autocatalytic.

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